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# Patogenicidad de *Beauveria bassiana* (bals) vuill., sobre el gusano barrenador de loche *Diaphania hyalinata* (Lepidoptera: Pyralidae) en laboratorio

## *Beauveria bassiana* pathogenicity (bals) vuill., the screwworm loche *Diaphania hyalinata* (Lepidoptera: Pyralidae) in laboratory

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## RESUMEN

Los hongos entomopatógenos son parásitos obligados que causan enfermedades en varias órdenes de insectos, constituyendo una alternativa ecológica en el Manejo de plagas en diferentes cultivos agrícolas. En el presente estudio se determinó la patogenicidad de *Beauveria bassiana*, para su aplicación potencial como agente de control microbiológico contra larvas de tercer estadío del barrenador del Loche *Diaphania hyalinata* (lepidoptera: pyralidae) unas de las principales plagas del cultivo de Loche. El aislamiento evaluado fue de aplicación directa de suspensiones $10^6$ y $10^{10}$ conidias/ml y registró una mortalidad en larvas de *Diaphania hyalinata,* entre 19% y 68% al cuarto día. En conclusión que la cepa *Beauveria bassiana* presenta un gran potencial como en el control microbiano de *Diaphania hyalinata.*

**Palabras clave:** control microbiano, *Beauveria bassiana*, *Diaphania sp.,* Loche.

## ABSTRACT

Diseases caused in many insects by parasites knowns as Entomopathógenic fungi composes an ecological alternative in the pest management in different agricultural crops. The patogenicity of the *Beauveria bassiana* was established in this present study as an important crop pest due to its potential application as microbiological control agent against the third instar larvae of the loche *Diaphania hyalinata* (lepidoptera: pyralidae) screwworm. The application of conidias suspensions of 106 and 1010/ml was evaluated in isolation, registering a mortality on *Diaphania hyalinata* larvae, between 19% to 68% in the fourth day. As a conclusion the *Beauveria bassiana* presents a great potential as a microbiological control of *Diaphania hyalinata.*

**Keywords:** microbiology control, Beauveria bassiana, Diaphania hyalinata, pumpkin plant.

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# INTRODUCTION

Loche cultivation (*Cucurbita moschata* Duch.) is characterized for being a creeper and climber plant, it has a rapid growth with leaves palmately lobed long stems not woody, hollow, angular, and have a tendency to produce roots inside the nodes.

The loche is a natural cucurbits from the department of Lambayeque, whose cultivation is limited in to the same province name and to Íllimo, Morrope, Tucume and Jayanca districts respectively; in the province of Chiclayo located between Reque and Monsefu and also in Pitipo district located in the province of Ferreñafe.

The cultivation of Loche has many adverse factors as: Blind chicken (*Phyllophaga sp*), whitefly (*Bemicia tabaci*), leafminer (*Liriomyza huidobrensis*) and the screwworn of fruits and stems (*Diaphania hyalinata* and *D. nitidalis*).

However, *D. Hyalinata* known as screwworm of fruits and stems can cause a defoliation, fail and rot from the Loche, including a commercial value, because they eat the leaves, flowers and fruits also fig the stem.

The presence of larvae in the fruit is recognized by one or several holes that segregate orange color feces.

This pest can be found along Canada, South America and the Caribbean according to Cave, (1995). The larva of *D. hyalinata* are pale green with two white stripes, they pass through five stages. A larvae adult is an attractive white butterfly with a small size, size (1.5 to 2 cm long and 2.5 cm of wing opening) wings are bordered by a dark strip of 1.5 mm wide. The abdomen ends in a white plume form. The egg hatches about 5 days and the larva delays around 9 to 14 days to become pupa, which at 6 or 7 days gives rise to the adult. Therefore, the life cycle lasts between 20 and 26 days, being able to give a considerable number of generations in the year.

The insecticides are a recommendation for a chemical control of this pest as: Cartap, Methamidophos, Methomyl, Spinosad, Fipronil, Tiodicarb for treatment before to the growing; mixtures: Methamidophos + Cyfluthrin effective for control, but it can affect the pollinators and fruit production.Concerning to the biological control, the Bacillus thuringiensis (strain 13) y *Metarhizium anisopliae* (Micotall strain) are recommended but the Beauveria bassiana, associated specie to control of Lepidoptero and beetles, has not been reported yet.

The principal symptoms caused by B. bassiana in sick insects before kill them are: loss of sensitivity, loss of appetite, melanization, paralysis, lack of coordination, and lethargy.

The increase from insect death is beneficial due to the sporulation and subsequent dispersal of the fungus, it allows more control towards the application (Estrada et al., 1997; Vargas, 2003; Malpartida, 2004).

On regards of this, the research was submitted, in order to study the strain of *B.basssiana* in the control of larvae of *D.hyalinata*, evaluating its pathogenicity,symptomatology, effect on feeding and morphological characterization.

The "screwworm Loche" might set an alternative efficient in microbiological control.

# METHOD

**Insect pest**

The larvae on the 3rd stage of *Diaphania hyalinata* were collected from a plant of Loche semi cultivated in the fences of productive area of rice Sector the Pancal, located District of Pisci, Province of Ferreñafe - Lambayeque.

There were not registration about bio pesticide application nor other type of pesticide. The collected larvae were placed in ventilated containers and carried out to the Cesar Vallejo University laboratory, the larvae with higher activity and feeding capacity were evaluated and selected to the study

***B. bassiana* strain**

In this study, the used strain was isolated and selected in the Laboratory of microbiology of the Cesar Vallejo University - Campus Chiclayo and propagated in a substrate of rice.

Reactivation **of B.bassiana**

The metabolic activity of the fungus aims to obtain a pure culture on a greater proportion using Agar Potato Dextrose (PDA), that it was extracted mycelium of the seed, planted in puncture and incubated at 28+- 0.5°C.

**Propagation and standardization of inoculum**

The suspension of *B.bassiana* was prepared in sterile solution of Tween 80 at 0.1 %, was used 0.1 ml suspension of the fungus, and incubated at 28°C until the formation of spores

Later, to obtain the spores an aqueous solution of Tween 80 at 0.1% was added. The spore suspension was placed in the culture flask. Then a chamber of Neubauer was standardized at the concentrations of 10 6, 108 and 1010 that were used as inoculum.

**Inoculation**

The sample consisted from 250 larvae of *D.hyalinata,* from Which 3 groups of 25 larvae were sample of the problem, and another group of 25 larvae were copies of a witness, making a total of 3 repetitions.

To the copies problem a suspension of spores in Tween 80 at 0.1% at concentrations of 106, 108 and 1010 spores/ml of *B. basssina* was inoculated by submersion and an aqueous solution of Tween 80 at 0.1% was inoculated to the witness.

**Evaluation of the activity entomopatógena *B. bassiana***

After inoculation, the onset of symptoms and/or signs of infection mitotic submitted by the larvae of the process in comparison with the witness was evaluated every 24 hours.

The observation was done daily by noting the appearance of symptoms such as loss of appetite, paralysis, pigmentation, or other, which occurred during the test, being the maximum time of observation of 10 days.

**Recovery and identification of *B. bassiana***

The aim of verifying the cause of death of larvae of *D.hyalinata* consisted on placing the larvae in a moist chamber and incubating at room temperature, the fungus was extracted once emerged with the help of a microbiological handle, then, was seeded in the test tube with PDA (Personal Development Analysis) for subsequent identification of the characteristic structures of B.bassiana.

## RESULTS AND DISCUSSIONS

**Pathogenicity, symptomatology and feed test.**

In table 1, shows the percentages of mortality obtained in this present study. According to what has been obtained, it is observed that among the process there were differences among themselves and with the witness which did not record any mortality during the test. In addition, that the mortality in the three processes was directly proportional to the concentration of conidia, found on the 4th day, the T3 was the most effective with 68 %.

Table 1
Average Percentage of mortality in larvae of D.hyalinata, on the 7th day after being inoculated with B. bassiana.

| Process | Concentration of conidia / ml | Mortality (%) |
|---|---|---|
| T1 | 106 | 19 |
| T2 | 108 | 46 |
| T3 | 1010 | 68 |
| WITNESS | 0 | 0 |

**Source:** statistical Processing of data.

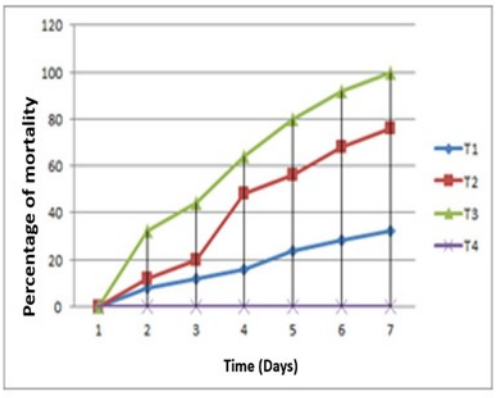


**Figure 1.** Cumulative mortality of larvae D.hyalinata according to the concentration employed.

In table 1 and figure 1, show the curve of mortality over time, caused such a concentration was different to those produced by concentrations of 108 and 106 conidia/ml, which reached 68%

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and 19% mortality respectively on the fourth day and 80% and 25% on the fifth day post-inoculation.

These results are approximated by other researchers, in other lepidopteran under similar laboratory conditions; and Malpartida, J.(2013) to a concentration of 108 conidia/ml were obtained in a mortality rate of 84% in larvae of Dione juno; while Quintana (2000), with 108 conidias/ml, achieved a 100% mortality in larvae of Rhyacionia buoliana Denis & Schiff (Tortricidae.).

For his part, Avalos, K. (2014), with concentacion of 106 conidia/ml and 107 conidia/ml, obtained 78.5% and 85% mortality on nymphs of Planococcus citri (Risso) at 72 hours after application respectively.

These results express the effectiveness of the fungus B.bassiana on larvae of lepidoptera and indicate that the concentration 1010conidias/ml with the highest effectiveness to get results in the biological control of pests.

As to the effect of the fungus on the food, it was obvious to observe the action on the larvae of D.hyalinata, from the 2nd day to decrease considerably his appetite, the 4th day no longer registers power.

Unlike the process, the witness maintained a growing trend with regard to the foliar consumption; except on the 4th day, which decreased, perhaps due to the aging of the larvae.

The linear trend is observed in the three processes, taking as an example the foliar consumption of the witness in a 100%, in figure 06.

It can be observed that a day after of the inoculation, in all process of the study, the consumption is almost the same; but from the 2nd day the exposed larvae to T3 present a significant reduction of the foliar consumption in 50% with regard to the witness on the 4th day there is no food registration.

Concerning the other concentrations, there is also a remarkable effect of the fungus, which causes a drop in power that reaches the fourth day up to 60% and 80% in processes P1 and P2, respectively. It demonstrates that the more concentration is, the greater will be the effect of the fungus, which causes increased mortality as a result.

The results were found with the information by Tefera & Pringle (2003) are similar, they reported that a concentration of 108 conidia/ml of B. bassiana in the second stage larvae of Aslan partellus (Pyralidae), food consumption was reduced by 70-85%, these researchers attribute the importance of this reduction due to the compensation for the slow action of fungi in its deadly effect on the insect.

According to Alves (1998) this low food consumption, perhaps it is due to the production of beauvericinas that act as inhibitors in the reactions of host defense, destroying the haemolymph and cells nucleus.

Table 2
Average foliar consumption during 4 days of larvae of D. hyalinata inoculated with 3 different concentrations of B. bassiana

| Process | Concentration of Coni- dias/ Ml | Foliar Consumption ($mm^2$) per day | | | | Total (cm) |
|---|---|---|---|---|---|---|
| | 106 | 1 | 2 | 3 | 4 | |
| P1 | 106 | 410 | 308 | 270 | 120 | 277 |
| P2 | 108 | 440 | 320 | 110 | 40 | 227.5 |
| P3 | 1010 | 412 | 200 | 10 | 0 | 155.5 |
| Witness | 0 | 430 | 450 | 460 | 330 | 417.5 |

**Source:** statistical Processing of data.

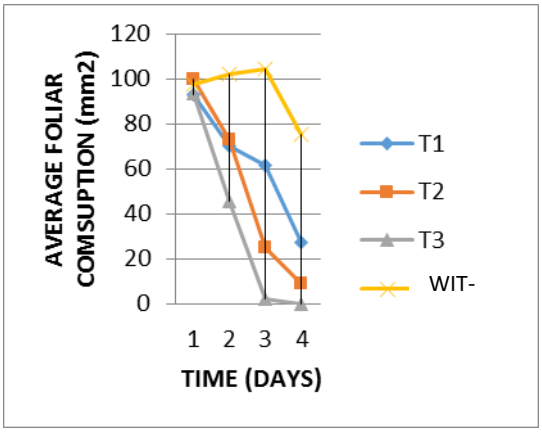


**Figure 2.** Average foliar consumption in mm2 of larvae of D. hyalinata inoculated with 3 concentrations of B. bassiana and the witness.

All dead and maintained in a humidity chamber larvae, presented mycelium between the second and third day after their death, confirmed the strain of B. bassiana, as the causative agent.

**Physiological characterization of the strain *B. bassiana***

The growth of the colonies of B. bassiana, in the majority of the plates, was expansive and homogeneous, in figure 07 displays the average radial growth during the 10 days, with linear trend progresses.

On average is 35 mm radial development.
The strain of B. bassiana, showed a high percentage of germination, with an average of 84.5%, demonstrating a high viability of their conidia.

The information reported by the authors showed a high similarity with our results in different growing medium, surpassing, the 90 % of germination Elosegui & Elizondo, (2010).

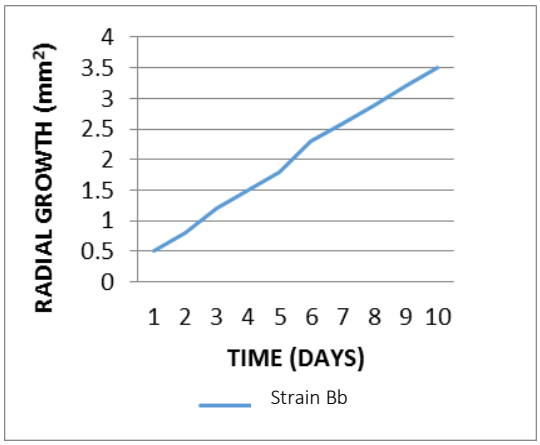


**Figure 3.** Radial growth of B. bassiana (concentration 108 conidia/ml) on the PDA, during 10 days of incubation.

**Figure 4:** Loche plants planted in the rice cultivation area.

**Figure 5:** Larvae of Diaphania sp in the third stage, selected for the test.

**Figure 6:** Dead larvae kept for 2 days in humidity chamber; it can be observed presence of mycelium.

**Figure 7:** colonies of fungi isolated in the midst PDA, 8 days of incubation at 25°C.

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## CONCLUSIONS

Under laboratory conditions, the strain of B. bassiana was pathogenic on larvae of D. hyalinata in 3rd stage, reaching 100% mortality with the concentration of 1010 conidia/ml; presenting a high capacity to reduce the consumption of food on the second day of application.

The Physiological characterization of the strain of B. bassiana, presents a good biological quality, because of it presents a close to 90% germination and a good growth medium.

The results of this studies, indicate that B. bassiana is an alternative of biological control, which must be included in the program of integrated management of D. hyalinata, especially if one takes into account the characteristics of the tegument.

However it is recommended to make previous studies, in field conditions to determine the most optimal concentration, as well as more favorable environmental conditions.

## RECOMMENDATIONS

The obtained results in B. bassiana are an alternative to control this plague, for which is suggested to carried out field testing at the various stages of the plague.

It is necessary to perform compatibility testing with chemicals, which were used in the Loche cultivating and were affected by this plague.

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